THE MECHANISM OF EVOLUTION[1]

IN THE LIGHT OF HEREDITY AND DEVELOPMENT

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V. The Cellular Basis of Ontogeny and Phylogeny

B. Mechanism of Heredity

2. *Share of Cytoplasm in Heredity*

THE evidences outlined in the preceding section demonstrate that the chromosomes contain the factors or genes of Mendelian inheritance, and it has been assumed by many investigators that the cytoplasm of the germ cells serves only as environment or food for the chromosomes and has nothing to do with heredity. Nevertheless there are certain characters of the embryo and adult that are derived directly from the cytoplasm of the egg and since these characters come from the mother and not from the father this may be called "maternal inheritance."

(a) *Differentiation of Cytoplasm of Germ Cells.*—The cytoplasm of both male and female sex cells is differentiated. A spermatozoon is perhaps as highly differentiated as any tissue cell; but these differentiations which have been built up during the late stages of spermatogenesis and which serve to bring the spermatozoon into union with the egg, disappear after that union either by the tail of the spermatozoon being left outside of the egg or by its disintegration or de-differentiation after it has entered. At the same time the egg ceases to form yolk while that which has been stored in the egg is gradually used up in the nourishment of the embryo. Consequently since these particular differentiations of the germ cells disappear after the union of egg and sperm it has generally been supposed that all cytoplasmic differentiations of these cells are wiped out at this time, and that the first differentiations of the new individual begin after fertilization in a wholly undifferentiated cytoplasm. However there is positive evidence that this is not the case and that many differentiations of the cytoplasm of the unfertilized egg persist and play an important part in embryonic differentiation (Fig. 22).

[1] William Ellery Hale Lectures before the National Academy of Sciences, Washington, April 16 and 18, 1917.

Spermatogenesis   *Oogenesis*

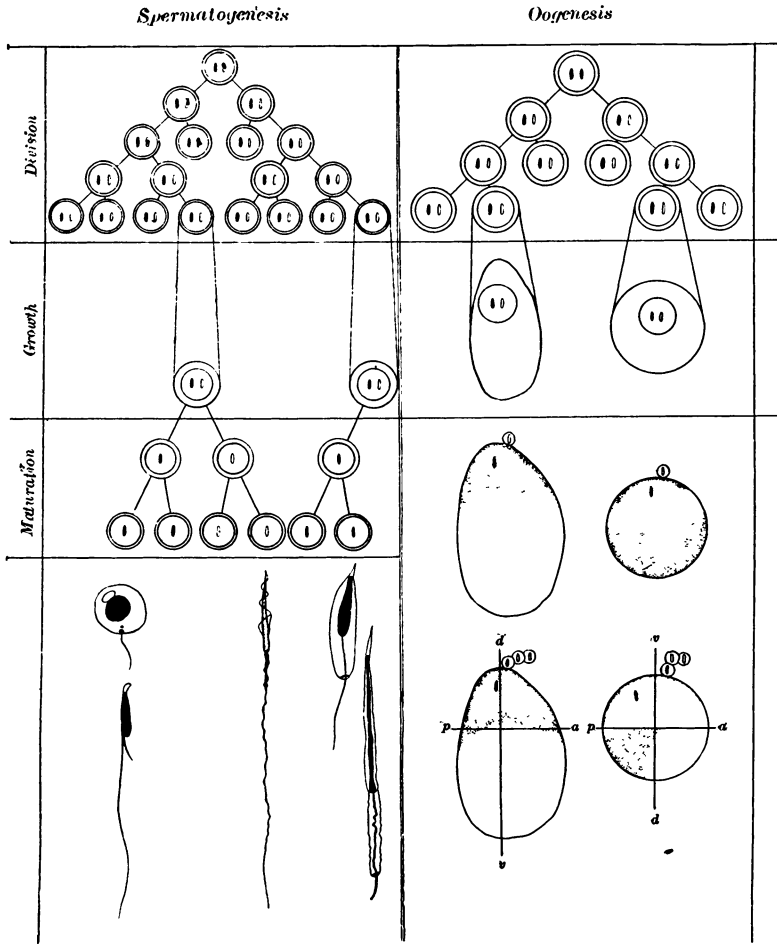
FIG. 22. DIAGRAM OF SPERMATOGENESIS AND OOGENESIS, showing Division, Growth and Maturation Periods and Final Stages of Spermatozoa and Ova. Two chromsomes, one from the father the other from the mother, are shown in each cell before maturation, only one after the reduction division. Three kinds of highly differentiated spermatozoa are shown and two kinds of ova. The differentiations of the spermatozoa are lost after they enter the egg, the polarity and symmetry of the egg persist and determine the orientations of development.

(b) *Egg Differentiations which persist in Embryo and Adult.* (1) *Polarity.*—The polarity of the egg invariably determines the polarity of the embryo and adult. In all animals the chief axis of the egg becomes the chief axis of the gastrula, and this becomes the chief axis of the adult in sponges and cœlenterates (protaxonia), or, as in all other metazoa (heteraxonia), this axis is bent on itself by the greater growth of the gastrula on its posterior side so that the chief axis of the adult is a modification of the gastrular axis. In either case the

polarity of the unfertilized egg determines the localization of developmental processes and ultimately the polarity of the developed animal.

(2) *Symmetry.*—In most animals the egg is spherical in shape and appears to be radially symmetrical, nevertheless observation and experiment show that such eggs are sometimes bilateral, as is probably the case in *Amphioxus,* ascidians, fishes and frogs. In the case of the frog's egg it was long believed that the plane of bilateral symmetry was determined wholly and exclusively by the path of the spermatozoon within the egg; more recently it has been shown by Brachet (1911) that primary bilateral symmetry is present before fertilization, though after fertilization the plane of symmetry may be shifted into the path of the spermatozoon. It is probable that all bilateral animals come from eggs which show a similar primary bilaterality and that this differentiation precedes fertilization. In cephalopods and some insects all the axes and poles of the developed animal are already recognizable in the egg before fertilization. Symmetry, therefore, as well as polarity, is derived from the egg and not from the sperm.

(3) *Inverse Symmetry (Asymmetry).*—In many animals the right and left sides of the body are not completely alike, and this is especially true of internal organs. This asymmetry is especially well developed in gasteropods in which certain organs of one side of the body are entirely lacking; some species or individuals have these asymmetrical organs on one side, others on the other side, and correspondingly the snail shell coils in a clock-wise direction in one case, an anti-clock-wise direction in the other. It was discovered by Crampton (1894) and Kofoid (1894) that in sinistral species the direction of certain cleavages of the egg (viz., the third to the sixth) was the reverse of the corresponding cleavages in dextral species and Conklin (1903) showed that the first and second cleavages also were in opposite directions in dextral and sinistral snails, and that these reversals of cleavage could be followed cell by cell to the reversal of symmetry in the larva. Consequently the inverse symmetry of these snails may be traced back through the later and earlier cleavage stages to the unsegmented egg itself which is inversely symmetrical in sinistral as compared with dextral forms.

(4) *Types of Egg Organization.*—The polar differentiation of an egg is manifested particularly in the localization of different kinds of materials in different parts of the egg. These materials may be inert pigment or yolk, but their localization

by the activity of the cytoplasm indicates a definite pattern of organization in the cytoplasm. This pattern of egg cytoplasm differs greatly in certain phyla, there being a cœlenterate type, an echinoderm type, a turbellarian-annelid-mollusk type, and a chordate type. The type of egg organization foreshadows the type of adult organization; in ascidians for instance distinct cytoplasmic substances are found in the egg in the same relative positions and proportions as the ectoderm, endoderm, mesoderm, notochord and nervous system of the embryo (Fig. 5, p. 495).

That the fundamental pattern of egg cytoplasm is not influenced by the spermatozoon is proved by the following facts:

(*a*) It exists before fertilization, or it appears so soon after that it could not have been caused by the sperm. (*b*) In heterogeneous fertilization the pattern of the egg is not changed by the foreign sperm. (*c*) Natural or artificial parthenogenesis demonstrates that this pattern exists in the absence of fertilization.

These as well as other facts such as the correspondence between the size of the egg and the size of the embryo (Morgan); the transmission of chromatophores and peculiarities of leaf coloration by the female and not by the male germ-cell in plants (Baur, Shull); the transmission in the egg cytoplasm of fat stains, chemical substances, immunizing bodies and possibly parasites, prove that,

at the time of fertilization the hereditary potencies of the two germ cells are not equal, all the early development, including the polarity, symmetry, type of cleavage, and the relative positions and proportions of future organs being foreshadowed in the cytoplasm of the egg cell, while only the differentiations of later development are influenced by the sperm. In short, the egg cytoplasm fixes the general type of development and the sperm and egg nuclei control only later differentiations (Conklin, 1908, 1915).

Ontogeny begins with the differentiation of the egg in the ovary and not at the moment of fertilization; at the latter time some of the most general and fundamental differentiations have already occurred. Indeed the cytoplasm of the egg is the more or less differentiated body of the embryo.

(*c*) *Is Inheritance through the Egg Cytoplasm Non-Mendelian?*—Whenever a character is transmitted as such through the egg cytoplasm and not as factors in the chromosomes of egg and sperm it is not inherited in Mendelian fashion. Thus if chromatophores are transmitted from generation to generation in the cytoplasm of the egg and are at no time influenced by the sperm, their mode of inheritance is non-Mendelian. If the

polarity, symmetry and pattern of the egg do not arise during oogenesis, but are carried over unchanged from generation to generation they are also non-Mendelian characters. With regard to the polarity of the egg, it is not certain whether it is transmitted in this manner or not; but its symmetry and pattern of organization are probably developed anew in each generation. However, Kunkel (1903) and Lang (1904) found that inverse symmetry is not inherited in Mendelian fashion and that it is doubtful whether it is inherited at all.

Most of the cytoplasmic differentiations of the egg and sperm arise during the genesis of those cells, just as in the case of tissue cells. Nerve cells and muscle cells differentiate under the influence of maternal and paternal chromosomes, and undoubtedly the same is true of most of the differentiations of egg and sperm; but while some of these egg differentiations persist in the new individual those of the sperm do not. Consequently, in each generation the egg contributes more than the sperm to ontogeny. There is cytoplasmic inheritance through the female only, but some of these cytoplasmic characters may be of biparental origin. If these characters are determined by genes in the chromosomes of cells from which the egg develops this is Mendelian inheritance or "preinheritance" though somewhat complicated by the fact that every ontogeny has its beginnings in the preceding generation, that is in the oogenesis, preceding fertilization; if they are not determined in this way but are carried from generation to generation in the cytoplasm the inheritance is non-Mendelian. The fact that certain differential factors must be located outside the chromosomes will be considered further in the section on the "Mechanism of Development."

3. *Specificity of the Germ Cells*

One of the greatest advances in biological knowledge during the past century is found in the increasing recognition that vital units are specific and that the larger units of organization are themselves composed of many minor units having their own individuality and specificity. All cells, or "vesicles" as they were called by Wolff (1759), were at first supposed to be alike, and protoplasm was regarded as a uniform substance in all organisms; it was, in the language of Dujardin (1841), "Substance glutineuse, parfaitement homogène, élastique, contractile, diaphane. On n'y distingue absolument ancune trace d'organization, ni fibres, ni membranes, ni apparence de cellulositié." When this view was no longer tenable this simplicity and homogeneity were ascribed to germinal layers, embryonic cells and germ cells. Even within recent years cleavage cells

were said by Driesch (1893) to be all alike, "like balls in a pile," and differentiations were said to appear only late in development as the result of environmental conditions. So great was the influence of the doctrine of Epigenesis! Finally when this was disproved, the various constituents of cells such as cytoplasm, nuclei, chromatin, chromosomes, etc., were supposed to be alike in all cells.

Now, on the other hand, we consider that there are as many different kinds of protoplasm as there are different kinds of cells,—that one chromosome differs from another and that even chromosomes and genes are individually distinct and peculiar. Perhaps the most general and important discovery as to the mechanism of heredity is that germ cells are as specific as developed organisms, that "the egg of a frog differs from the egg of a hen as much as a frog differs from a hen"—indeed that just as every sexually reproduced animal or plant differs more or less from every other one so every germ cell differs more or less from every other germ cell.

Considering the vast numbers of germ cells which exist, their minute size and the difficulty of directly determining differences between them this statement may well challenge criticism and call for proof. And yet there is no gainsaying the fact that hereditary differences are due to germinal differences, and although no man can actually see the differences between the germ cells of different races or sometimes even of different species or genera, the results of development clearly demonstrate that such distinctions exist. In this case as in so many others physiological indicators are more delicate than morphological ones.

We have physiological proof that different chromosomes of the same cell differ in hereditary value and that different germ cells from the same individual frequently differ in hereditary constitution. In the separation of bivalent chromosomes at the reduction division each chromosome may go into either of the two daughter cells and the number of different combinations of chromosomes which may occur in the gametes is $(2)^n$, in which n is the number of bivalent chromosomes. Thus if there are 24 bivalent chromosomes, as in man, the possible permutations of these in the germ cells is 2^{24} or 16,777,216 and the possible number of different *combinations* of maternal and paternal chromosomes in the fertilized egg is this number squared or approximately three hundred thousand billions, though the actual number of *genotypes* is only 3^{24} or about thirty thousand billions, assuming that every chromosome differs hereditarily from every other one. Consequently it is not surprising that

successive children of the same parents are rarely if ever alike, since their chromosomal constitution is rarely if ever the same.

But the case for the specificity of germ cells is even stronger than this for there is reason to believe that particular chromosomes are not always composed of the same chromomeres (see p. 289) and the possible permutations of the many chromomeres of each chromosome furnishes an almost infinite number of combinations of units which are still visible with the microscope. Finally when we consider the possible combinations of still smaller units such as genes it is probable that every separate oosperm and every individual which develops from it is absolutely unique.

This conception of the specificity of gametes and zygotes sets the whole problem of the mechanism of heredity in a clear light. Unique individuals come from unique germ cells. Hereditary resemblances and differences in adult organisms are correlated with resemblances and differences in the germ cells from which they came.

C. MECHANISM OF DEVELOPMENT

Development is progressive and coordinated differentiation by which the egg becomes the embryo and ultimately the adult organism. It is concerned with the manner in which the egg cell gives rise to different kinds of embryonic and tissue cells and with the way in which the latter form various cell products such as muscle and nerve fibrils, cartilage and bone. Development is also concerned with the coordination and integration of these differentiations so that an orderly arrangement of parts results. The mechanism of development, like the mechanism of heredity, is a cellular problem and it can be discovered only by the study of the cellular differentiations and integrations of development. But development, or the *transformation* of germinal units into developed characters, is a much more complex process than heredity, or the *transmission* of germinal units from one generation to the next, and at present relatively little is known as to the precise mechanism of such developmental transformations.

1. *Stability of Chromosomes in Development*

One of the most striking facts in ontogeny is that amid all the remarkable changes and differentiations which are taking place in cells their chromosomes remain almost entirely unchanged.

(*a*) *In Egg and Sperm.*—This is true of all kinds of cells but it is nowhere more striking than in the egg and sperm. These cells are notably unlike each other; the spermatozoon is one of the smallest of all cells, the egg is one of the largest, and correspondingly the entire nucleus of the spermatozoon is often smaller than a single chromosome of the egg; virtually all of the cytoplasm of the sperm is converted into a complicated locomotor apparatus whereas the cytoplasm of the egg is abundant, relatively undifferentiated and contains much yolk. It is certainly no exaggeration to say that the sperm and egg differ as much as muscle and nerve cells or as connective tissue and gland cells. And yet in spite of this great difference between the two germ cells their chromosomes are so much alike after they have united that it is usually impossible to distinguish them. The extreme differentiation of the spermatozoon has not in any way changed its chromosomes for they issue from it exactly as they went into it, the same in number, shape and even in genetic constitution in spite of the fact that they have been compressed into one of the smallest of all nuclei in one of the most highly differentiated of all cells.

(*b*) *In Tissue Cells.*—The chromosomes of tissue cells are, in many cases, precisely like those of the oosperm. The differentiations which the cell body has undergone do not usually modify the chromosomes. This is especially true of embryonic cells where mitoses are more abundant and more easily studied than in fully developed tissue cells. In Orthoptera, according to McClung, not only the number of all the chromosomes but even the shape of each particular chromosome is constant for all cells of a given individual, though they may differ slightly in different individuals of the same species. Hoy found that the numbers and shapes of chromosomes in embryonic muscle, nerve, gut and connective tissue cells of certain insects were the same as in the fertilized egg.

On the other hand, Miss Holt found that the number of chromosomes in the alimentary tract of larval mosquitoes might vary from 6 to 72 but always in multiples of 3, the haploid number in this species. She concludes therefore that these variations in number are due either to a double splitting of each chromosome in certain mitoses or more probably to the failure of the cell to divide after the chromosomes have divided. The latter is a phenomenon which not infrequently occurs in cells subjected to experimental conditions. Hance found in the pig that the somatic chromosomes of many cells are more numerous than the diploid number but he has proved that

this is due to fragmentation of chromosomes, the fragments when added together merely equalling in length the original chromosome. Such fragmentation of chromosomes in somatic cells was first observed by Boveri in the case of *Ascaris megalocephola* var. *univalens* (Fig. 23). Here the diploid number of
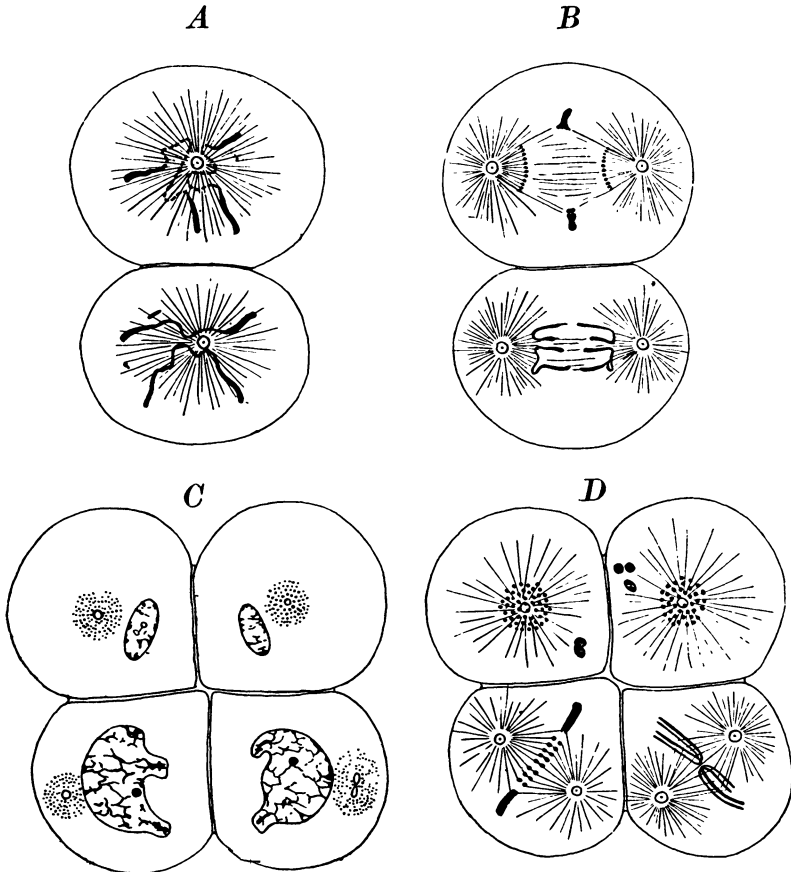

FIG. 23. DIFFERENTIATION OF CHROMOSOMES OF SOMATIC CELLS IN *Ascaris megalocephala*, var. *univalens*. *A* and *B*, Second cleavage mitosis showing the chromosomes entire in the lower cells (germ track) ; in the upper (somatic) cells their ends are thrown off and the remainder breaks up into many small chromosomes. *C*, 4-cells, the upper two (somatic cells) containing small nuclei derived from the small chromosomes. *D*, Third nuclear division, showing the somatic differentiation of the chromosomes in all the cells except the lower right one, which alone is in the germ track and will give rise to sex cells. (After Boveri.)

chromosomes is 2 but in somatic cells more than 30 small chromosomes (or chromomeres) are present, the total volume of which is even less than that of the 2 original chromosomes since the ends of these chromosomes are cast off and dissolved in the cytoplasm (Fig. 23, *C*, *D*). Boveri has proved by exper-

iments that whether chromosomes fragment or not depends upon whether they lie in a particular field of differentiated cytoplasm and consequently that this is due to the influence of cytoplasm on the chromosomes, and not the reverse.

In conclusion, the chromosomes of tissue cells, as well as those of germ cells, are usually unmodified by the differentiations of the cell body. In other cases these chromosomes may be fragmented or possibly otherwise modified by the differentiations of the cell body. But there is no evidence that the differentiations of these chromosomes are primary and that they cause the differentiations of the cell body as Weismann assumed.

2. *Differentiation of Cytoplasm in Development*

The cytoplasm is the chief, perhaps in some cases the only, seat of differentiation in the developing organism. Practically all the differentiations of cells, tissues, organs and persons are differentiations which arise (1) by transformations of cytoplasm (autoplasmatic differentiation) or (2) as secretion products of cytoplasm (apoplasmatic differentiation).

a. *The Structure of Cytoplasm in General.*—All students of the cell now agree that protoplasm is composed of a more fluid and a more solid portion, though there is much difference of opinion as to the form of each of these and their relations to each other, as is shown by the various theories of the "structure of protoplasm." The more fluid part is often called enchylemma or cytolymph, the more solid part spongioplasm or kinoplasm; the latter forms some sort of a framework, in the form of fibers, or a net-like or a foam-like structure while the former fills the interstices which are left. Recent experiments on cells and protoplasm indicate that these two portions may vary in consistency at different phases of cell activity, such variations being perhaps in the nature of reversible sols and gels.

But whatever the relation may be between these portions of protoplasm, experiments in which cells are subjected to strong centrifugal force indicate that the framework of the cell is a viscid, elastic, contractile gel which is more rigid at certain phases of the cell cycle than at others, depending probably upon its water content, and that within this gel are included water, oil, yolk, pigments, granules and other products of differentiation.

In addition to this general framework of spongioplasm there is present in many cells, especially at the time of division, a special structure, the mitotic figure or amphiaster, consisting probably of an elastic, contractile gel which takes the form of

a spindle with star-shaped radiations at its two poles. This amphiaster is undoubtedly an apparatus for the division of the nucleus and cell body, but we do not know exactly how it functions, though it probably brings about intra-cellular movements, together with segregations and localizations of different cell substances.

b. Formation of Different Substances in Cytoplasm.—In the course of development different substances are formed in different parts of cells or in different cells. The exact manner in which these substances are formed is a matter of great interest in connection with the mechanism of differentiation and development. Unfortunately our knowledge upon this point is very incomplete. The general evidence seems to favor the view that the nucleus cooperates in the formation of the various differentiation products which appear in the cytoplasm; in general these substances are not formed when the nucleus is absent though once differentiation of cytoplasmic substances has begun it may go on for a time in the absence of the nucleus. The manner in which the nucleus cooperates in differentiation has been variously interpreted by different authors. Weismann assumed that in mitosis there was a differential distribution of the nuclear inheritance factors to the different cells and that in this way the differentiations of development were to be explained. The evidence is all against this view as Boveri and others have shown. In the first place the splitting of chromosomes is never differential and the mitotic apparatus is unsuited to a differential separation of daughter chromosomes. Only in the separation of whole chromosomes which had previously united in pairs, as in the maturation divisions, is such a differential separation possible. In the second place the experiments of Driesch and O. Hertwig on compressed frog's eggs and of Conklin on Ascidian eggs proves that nuclear divisions are not differential, but that cytoplasmic divisions often are. Finally the differentiation of the somatic chromosomes of *Ascaris* (Fig. 23) is due to the fact that they lie in a field of peculiar cytoplasm as Boveri has shown; consequently such chromosomal differentiations are the result of cytoplasmic differentiations and not their cause.

(1) *"Intracellular Pangenesis."*—Under this name deVries (1889) proposed a hypothesis of the way in which the nucleus controls differentiation which nearly meets present requirements and fits present knowledge. He suggested that particles which he called "pangenes" and which he regarded as enzymatic in character escape from the nucleus into the cell body

where they form the entire living protoplasm. Most of the nuclear pangenes are inactive but they may be activated by age or outer circumstances. Every nucleus contains a full set of pangenes and there is no differential division of them in mitosis. The pangenes which escape from the nucleus must be transported to different parts of the cell and segregated at the right places and this transportation and localization is brought about by the streaming of the protoplasm.

In its main features this hypothesis is acceptable to-day. However deVries's conception that "every inherited character has its particular kind of pangene," has suffered the same fate as the more recent view that "determinants," "determiners" or "inheritance factors" are the germinal representatives of developed characters. These are not the germs of developed characters any more than oxygen and hydrogen are the germs of water; on the other hand they are only specific causes which, acting in conjunction with other causes, produce specific results.

Furthermore it is not now believed that genes or pangenes maintain their identity in the cytoplasm and that the entire cytoplasm is composed of them. It is not even known that genes escape *as such* from the nucleus into the cytoplasm but it is known that there is an escape of nuclear substances into the cytoplasm and that as a result of syntheses of these with portions of the cytoplasm new substances are formed which were not present before.

(2) *Escape of Nuclear Substances into Cell-body*

(*a*) *At Mitosis.*—Every cytologist is familiar with the fact that various nuclear substances escape into the cell-body. One of the most striking instances of this occurs during mitosis when the nuclear membrane is dissolved and its contents are set free into the cell. In some cases the entire volume of nuclear materials which are thus set free has been estimated to be five-hundred times as great as the volume of the chromosomes which give rise to the daughter nuclei. Among the nuclear materials which thus escape are nuclear sap, linin, nucleoli and oxychromatin. The last is probably derived from the chromosomes and it takes part in the formation of the "sphere substance" which surrounds the centrosomes. This sphere substance is differentially distributed to different cleavage cells as for example in the case of Crepidula (Conklin, 1902), and it probably cooperates in the differentiations of these cells.

The immediate results of the escape of nuclear materials into the cytoplasm are both varied and striking. At once there

is greatly increased oxidation with the formation of carbonic acid, and the cell which was before relatively stable and resistant becomes unstable and peculiarly liable to injury.

Another result is the rapid formation of astral rays and spheres around the centrosomes; indeed very much of the cell cytoplasm may be transformed into these structures following the escape of nuclear material during mitosis.

Another notable result of this escape of nuclear material is found in the movements which are set up in the cell and which lead to the separation of daughter chromosomes, the orientation of spindles, the localization of different cytoplasmic materials and finally the division of the cell-body. All of these movements, which may be collectively known as karyokinesis and cytokinesis, start with the escape of nuclear material into the cytoplasm.

(*b*) *Chromidia.*—R. Hertwig and his associates maintain that chromatin may escape through the nuclear membrane into the cell-body during resting stages. Such escaped chromatin forms chromatic granules which they call "chromidia" and which they believe take part in the differentiation of various intracellular structures, such as skeletal, muscle- and nerve-fibrils, secretion granules, pigment and the Nissl-bodies of nerve cells. However it is not certain that chromidia are derived from escaped chromatin.

(*c*) *Mitochondria.*—Other observers, particularly Meves and Duesberg, have described granules, rods or threads in the cytoplasm which are known by the general name of "mitochondria" and which appear to take part in the differentiations of specific structures. Meves and Duesberg regard these as purely cytoplasmic bodies which have the power of growth and division. Hertwig and his school hold that they are derived from chromidia, and therefore in the beginning from chromatin; more probably they are new formations caused by the action of chromatin on cytoplasm.

(3) *Cytoplasmic Differentiation*

One of the simplest cases of differentiation is found in the formation of secretion products within cells, such as yolk, oil, and zymogen granules. These usually appear as minute granules or droplets which then grow in size until they more or less completely fill the cell. Whether they are products of destructive or of constructive metabolism is not altogether clear; probably in some cases they are the former, in others the latter.

Yolk and zymogen begin to form in the vicinity of the

nucleus and in many cases out of a granular mass which is either chromidia, mitochondria or the granular substance surrounding the centrosomes and known as "sphere-substance." In some cases, perhaps in all, the granular body known as a "yolk nucleus" is sphere-substance derived from the interaction of nucleus and cytoplasm; according to the Hertwig school zymogen granules are always derived from chromidia and pigment usually comes from the same source.

Intra-cellular fibrils, such as skeletal, muscle- and nerve-fibrillæ, are also derived from chromidia, according to Goldschmidt, and hence are formed in large part by substances derived from the nucleus. On the other hand, Meves and Duesberg maintain that such intra-cellular differentiations are derived from mitochondria which are purely cytoplasmic in origin. The more probable view is that mitochondria are formed by the interaction of nucleus and cytoplasm, and that all other cellular differentiations are formed in the same way; and if all these cytoplasmic differentiations are produced by the action of chromatin on cytoplasm, the chromatin is only one factor in their origin.

As a result of all these observations it is impossible to avoid the conclusion that the nucleus is intimately concerned in differentiation, and the mechanism of the "nuclear control" of the cell is at least suggested by the escape of chromatin into the cytoplasm and the formation there of various differentiation products such as mitochondria, sphere substance, fibers, granules, etc.

(c) *Segregation and Isolation of Different Substances.*— Embryonic differentiation involves not only the formation of different substances in cells, but also their segregation in particular parts of cells and ultimately, in most cases, their isolation in different cells. Such segregation and isolation are seen especially well in the cleavage of the egg. By the flowing movements of cytoplasm during mitosis the various substances in the cells are oriented and sorted and by the formation of division walls between daughter cells these substances become permanently isolated. Such segregation and isolation of different cytoplasmic substances is certainly one of the most important functions of cleavage.

(1) *Differential and Non-Differential Cell Divisions.*—Cell dixisions are plainly of two kinds, differential in which the daughter cells are unlike in size or contents, and non-differential in which they are alike. Both of these occur in the cleavage of the egg but only the former contributes directly to

embryonic differentiation. Given a cell which is not homogenous, or in which every radius is not like every other one, it follows that cell divisions will be differential or non-differential depending upon the position and direction of the cleavage planes. Under normal conditions these planes are constant in position and they follow one another in a definite sequence just as do all processes of development. Consequently the pattern and character of the cleavage and its relation to differentiation is nearly constant for each species. Any explanation of the causes of differential cell-division must account for the localizations of different materials in cells and for the orientation of the planes of cell division.

(2) *The Orientations of Development.*—All of the orientations of development find their earliest visible expression in the polar differentiation of the egg. This polarity determines not only the polarity of cleavage cells, embryos and adults but it is also causally related to the direction of movement of the germ nuclei and of cytoplasmic substances, and consequently to the type of symmetry and the pattern of localization, as well as to other orientations of development. The problem of the causes of these orientations is perhaps the greatest problem of embryogeny.

Experiments with eggs subjected to centrifugal force indicate that pigment, oil, yolk and other inclusions are passively localized in certain parts of the cell and that the substance in which polarity persists is the elastic, contractile spongioplasm, which differs in structure or consistency at different poles and at different stages of the cycle of division. Protoplasmic flowing may be best explained as the result of the contractility of the spongioplasm, and the definite localization of inclusions, mitotic spindles and division planes, as caused by the polar differentiations of the spongioplasm. This polar differentiation of the spongioplasm persists and to it are to be referred in the last analysis many if not all of the orientations of development.

(3) *The Chromosome Theory of Heredity Applied to Embryonic Differentiation.*—According to the chromosome theory of heredity the inheritance factors are located in the chromosomes, and the cytological evidence shows that chromosomes always divide equally and presumably every cell of an individual contains the same kinds of chromosomes and the same kinds of inheritance factors. How then is it possible to explain embryonic differentiation? How can identical factors give rise to different products in different cells?

This is evidently due to the fact that while the division of chromosomes is non-differential, that of the cell body is often differential and the same chromosomes and genes acting upon different kinds of cytoplasm will produce different results (Fig. 10, p. 54). But differential cell-division is the result of definite movements in the cytoplasm, of definite orientations of spindles and cleavage planes, and ultimately of a definite polarity and symmetry of the cytoplasm. There is abundant evidence that these cytoplasmic orientations are not the immediate results of chromosomal activity and even if some of them may be the remote results of such activity it is logically impossible to place all the differential factors of development in non-differentiating genes.

On the other hand if embryonic differentiations are produced by the interaction of chromatin and cytoplasm, and if the chromatin does not undergo differentiation, it follows that some of the differential factors of heredity and development must be located in the cytoplasm. Such factors would probably not be genes and would not be transmitted in Mendelian fashion, but they would need to be present in the cytoplasm from the very beginnings of ontogeny. They need not be numerous—in fact they are probably few in number—but they are absolutely indispensable to development. If a few orientating differentiations such as polarity and symmetry are present in the cytoplasm at the beginnings of ontogeny all other differentiations of development can be explained as due to the interaction of non-differentiating genes on different parts of this cytoplasm, but there is no mechanism by which embryonic differentiations could come from the action of non-differentiating genes on a homogeneous cytoplasm. The genes or Mendelian factors are undoubtedly located in the chromosomes and they are sometimes regarded as the only differential factors of development, but if this were true these genes would of necessity have to undergo differential division and distribution to the cleavage cells; since this is not true it must be that some of the differential factors of development lie outside of the nucleus and if they are inherited, as most of these early orientations are, they must lie in the cytoplasm.